# Postharvest sweetening of potato seed tubers and effects on their yield performance

Jiang, J. University of Wisconsin-Madison
Eshel, D. Agricultural Research Organization

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Abstract

Potato (*Solanum tuberosum* L.) is one of the most important food/vegetable crops in the United States and the highest gross value crop in Israel. Sweetening of seed tubers during cold storage is a serious problem because it affects the number of plant stems and impacts daughter-tuber sizes. Israeli farmers import ~30,000 tons of seed tubers annually because it is nearly impossible to prevent oversprouting and branching in cold storage of seed tubers harvested in the summer and to hold the qulity of the seed tubers until the following winter. Growing certain potato cultivars in the cold, northern United States presents the opposite challenge: long dormancy and a low number of stems growing from the seed tubers. Our original research objectives were: 1. To determine the role of sugars vs. phytohormones originating from tuber parenchyma in signaling etiolated stems to branch, using grafting followed by stem-sap analysis; 2. To determine whether *VInv*-overexpressing lines produce less stems in response to branching inducers; 3. To edit the *VInv* gene, using CRISPR/Cas9, to modify stem numbers in non-transgenic commercial cultivars. Our working hypothesis was that the parenchyma sweetening induced by storage temprature is a key factor controlling stem number and branching. Although we invested a significant amount effort to collect stem sup, including building a specific device that allows us to connect a stem to the HPLC, we were not able to determine whether the very low volume of sup that we could collect is from the xylem, phloem or tissue crush. Therefore, we designed an alternative approach: feeding detached stems with sugars, sugars analogs, and radiolabeled sugars. Our results indicate that sucrose feeding to detached stems promotes accumulation of cytokinins (CK), and enhance the production of vacuolar invertase (VInv), an enzyme contributing to sugar sink strength. The sucrose effect was suppressed by inhibitors of CK synthesis and perception. CK supply to detached stems can induce bud outgrowth and VInv activity in the absence of sucrose in the growth medium. CK-induced bud outgrowth was suppressed in the background of *vinv* mutants, generated by genome editing. Altogether, our results revealed a branching-promoting module, suggesting that sugar-induced lateral bud outgrowth is partially promoted by the induction of CK-mediated VInv activity. Based on the knowledge gained from this project, we reduce dramatically the sweetening in cold storage by CRISPR/Cas9 knockout of VInv of comertial cultivars. We produced VInv overexpression transgenic lines wich will serve as an importat research tool in future study. Our combined expertise allowed us to discocer new aspects of potato sweetening during cold storage and novel genome-editing protocols for improved seed-tuber storage and for sprouting control. Identification of key mechanisms associated with sweetening is also relevant for the development of new strategies or treatments to alleviate sugar accumulation postharvest. The byproducts of this research may mitigate health concerns by allowing the development of potato cultivars that will have reduced contents of the acrylamide precursors, glucose and fructose, in cold-stored tubers.

## Summary Sheet

## Publication Summary

| PubType | IS only | Joint | US only |
|---|---|---|---|
| Reviewed | 0 | 2 | 0 |

## Training Summary

| Trainee Type | Last Name | First Name | Institution | Country |
|---|---|---|---|---|
| Ph.D. Student | Salam | Bolaji Babajide | ARO, The Volcani Center | Israel |
| Postdoctoral Fellow | Aruchamy | Kalaivani | ARO, The Volcani Center | Israel |
| Postdoctoral Fellow | Braz | Guilherme | MSU | USA |
| Postdoctoral Fellow | Zhao | Hainan | MSU | USA |
| Postdoctoral Fellow | Fang | Chao | MSU | USA |

Contribution of collaboration

**Details of Cooperation**

This project was truly synergistic, relying on the very different and complementary research expertise required. The Israeli PI, Dr. Eshel, has a great deal of experience and knowledge in physiological and molecular aspects of postharvest biology of potato tubers. The potato CRISPR/CAs9 techniques being used in the proposed are extensively used in his laboratory and shared with the US lab.

The US Co-PI, Prof. Jiang, is a potato geneticist with much experience and knowledge in potato breeding and genomics. The *VInv*-silenced and overexpressing lines to be used in this study have been developed and characterized in his laboratory. Thus, reagents, protocols and samples were and will be delivered between the US and Israel, as done successfully in the last 3 years. It is clear this study involves an active and true collaboration between the two teams, as either group alone could not perform it.

Achievements

*1. Determine the role of sugars vs. phytohormones in signaling etiolated stems to branch*

**Sucrose induces lateral bud (LB) elongation better than hexoses** - In the first year we showed that sucrose and its hydrolytic products induce stem branching in a dose-response manner in etiolated potato stem (Salam et al., 2017). To distinguish the sugar effects on bud burst vs. bud elongation, we conducted a time course study of the differential effects of sucrose or a mix of glucose and fructose (hexoses) on the number of branches and lateral bud elongation. Both sucrose and hexoses induced branching and lateral bud elongation (Appendix, Fig. 1). Water and the sugar alcohol and osmotic agent sorbitol, which can be imported but is not generally (or only slowly) metabolized by plant cells, were unable to induce branching and elongation (Appendix, Fig. 1). Sucrose and hexoses yielded similar branching, however, lateral bud elongation was significantly higher during the nine days of sucrose feeding compared to hexose feeding.

**Sucrose and hexoses translocate to the stem and penetrate the LB**- To test whether sucrose and hexoses are translocated into the LB itself or only to its base (node), we fed labeled sugars ([U-$^{14}$C]sucrose, or [U-$^{14}$C]glucose and [U-$^{14}$C]fructose) to the bottom of detached stems. After 2 h of incubation with either sucrose or hexoses, we detected radioactivity at the node and inside the lateral bud (Appendix, Fig. 2). While these results indicated translocation and entry into the lateral bud, it was not possible to determine whether the radioactivity measured in the buds was due to the movement of glucose and fructose, or to their reconversion to sucrose.

**Sucrose triggers CK accumulation prior to stem-branching initiation-** We tested whether CK is involved in the sucrose-induced bud outgrowth by quantifying CK accumulation in the stem node following sugar feeding of etiolated stems. The levels of intermediate (zeatin riboside) and active (zeatin) CK forms increased following feeding with sucrose (Appendix, Fig. 3). The response of zeatine level to hexoses or sorbitol feeding was weaker than its response to sucrose (Appendix, Fig. 3), revealing that sucrose is more potent in inducing CK accumulation. Supplying etiolated stems with synthetic CK 6-benzylaminopurine (BAP) led to a dose-dependent increase in branching and bud elongation, similar to the effect of sucrose feeding (Appendix, Fig. 4). Feeding with a mixture of BAP and sucrose significantly increased branching and lateral bud elongation compared to using each treatment alone (Appendix, Fig. 4).

Achievements

To determine whether CK mediates the effect of sucrose on stem branching, we supplied inhibitors of CK synthesis (lovastatin) or perception (PI-55, LGR-991) to etiolated stems with sucrose. The effects of sucrose on stem branching and lateral bud elongation were completely suppressed by these inhibitors (Appendix, Fig. 5). LGR-991 and PI-55 caused repression of bud outgrowth that could not be overcome by BAP application. These results suggest that sucrose requires CK to induce bud burst and elongation.

*2. The role of Vacuolar invertase (VInv) in tuber/stem branching*

**Sucrose induces the expression and activity of VInv prior to lateral bud elongation-** Since hexoses induce stem branching, we hypothesize that bud burst induced by sucrose is mediated by the activity of invertases, the key enzymes involved in sucrose degradation, in the developing bud. Etiolated stems were detached and fed with sucrose, hexoses, or sorbitol for 24 h, and the transcription and enzyme activity of VInv and cell-wall invertase (CWInv) were determined. After 2, 8, and 10 h of sucrose feeding, the *VInv* transcript level in the third lateral stem bud was significantly higher compared to feeding with the other sugars. In contrast, *VInv* expression was not different between hexoses and sorbitol feeding for >24 h (Appendix, Fig. 6A). The expression levels of seven *CWInv* genes were not significantly upregulated in response to sucrose feeding, as compared to hexoses or sorbitol feeding. VInv and CWInv activities were enhanced in the lateral bud of sucrose-fed stems as early as 2 h after feeding and remained significantly higher until 24 h after feeding (Appendix, Fig. 6 B and C). Altogether, these results show that invertases are associated with sucrose-promoted bud outgrowth. Since VInv was explicitly regulated in both transcript level and enzyme activity, we decide to focus our further experiments on this enzyme.

**VInv is involved in branching-** To investigate the role of VInv in sucrose-induced stem branching and bud elongation, we developed two *VInv*-CRISPR/Cas9 mutantion lines (*vinv-7* and *vinv-8*), which showed low VInv-activity levels . We compared the effects of sucrose feeding between wild-type (WT) plants and *VInv* mutants. The number of sucrose-induced branches was substantially reduced in *VInv*-mutant lines compared to the WT, suggesting a role for VInv in the sucrose-induced branching (Appendix, Fig. 7A). There was also a significant reduction in sucrose-induced lateral bud elongation in the *VInv* mutants (Appendix, Fig. 7B). These results were associated with the low VInv activity in the *vinv-7* and *vinv-8* lines fed with sucrose as compared to the WT (Appendix, Fig.

Achievements

7C). These results suggest that VInv activity is important for sucrose-induced lateral bud burst and elongation.

**VInv Activity is Induced By Sucrose and CK-** Since a mixture of sucrose and CK inhibitors yielded no branching, we hypothesize that VInv activity is affected by CK, or that CK is required by VInv-mediated branching. To test these hypotheses, we analyzed the impact of CK inhibitors on CK- and sucrose-induced VInv activity. CK inhibitors reduced effects of both sucrose and BAP on VInv activity (Appendix, Fig. 8), indicating that both sucrose and CK can trigger VInv activity, and that the impact of sucrose on VInv activity is partially dependent on CK.

To test whether VInv mediate the effect of CK on branching, we investigated the impact of BAP on stem branching in *VInv* mutants. The *vinv7* and *8* mutants were treated with 100 or 200 µM BAP for 15 days in the dark. Both lines showed reduced branching (Appendix, Fig. 9A, B) and elongation (Appendix, Fig. 9C, D) in response to BAP, showing significant differences after 15 days. These results support the notion that CK induces branching in part through induction of VInv activity.

**Overexpression of VInv-** Both groups developed constructs for overexpression study for the *VInv* gene. These constructs were used to transform potato cultivar Katahdin (at MSU) and Desiree (at ARO). Ten positive lines were developed for 'Katahdin' and transferred to the greenhouse (16 h 24 °C/8 h 16 °C light/dark). At the first growth cycle, we didn't detect any significant changes as related to stem branching or chip color change after frying (Appendix, Fig. 10). The transgenic lines are in second cycle of groth and will be further characterized.

In summary, our comprehensive experiments have revealed that sucrose induces bud growth and elongation better than its moieties. Sucrose, but not hexoses, activates CK accumulation. Elevated sucrose (directly and indirectly) and CK are associated with higher invertase activity, which contributes to bud outgrowth. The CK/VInv pathway plays a role in increasing the capacity of sugars to promote bud outgrowth. This property is potentially important in the competition between axillary buds. Further studies are needed to determine how this mechanism affects the establishment of shoot architecture in plants.

References: **Salam BB, Malka SK, Zhu X, Gong H, Ziv C, Teper-Bamnolker P, Ori N, Jiang J, Eshel D** (2017) Etiolated stem branching is a result of systemic signaling associated with sucrose level. Plant Physiology **175:** 734-745

Contribution of collaboration

**Details of Cooperation**

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## Publications for Project IS-5038-17C

| Status | Type | Authors | Title | Journal | Vol:pg Year | Coun |
|---|---|---|---|---|---|---|
| Accepted | Reviewed | *40. Salam B. B., Barbier F., Danieli R., Teper-Bamnolker P., Ziv C., Sp?chal L., Aruchamy K., Shnaider Y., Leibman D., Shaya F., Carmeli-Weissberg M., Gal-On A., Jiang J., Ori N., Beveridge C. and Eshel D.* | Sucrose promotes stem branching through cytokinin | ***Plant Physiology*** | in press : 2021 | Joint |
| Published | Reviewed | *Bolaji Babajide Salam, Siva Kumar Malka, Xiaobiao Zhu, Huiling Gong, Carmit Ziv, Paula Teper-Bamnolker, Naomi Ori, Jiming Jiang, and Dani Eshel* | Etiolated Stem Branching Is a Result of Systemic Signaling Associated with Sucrose Level | ***Plant Physiology*** | 175 : 734-745 2017 | Joint |

Figures

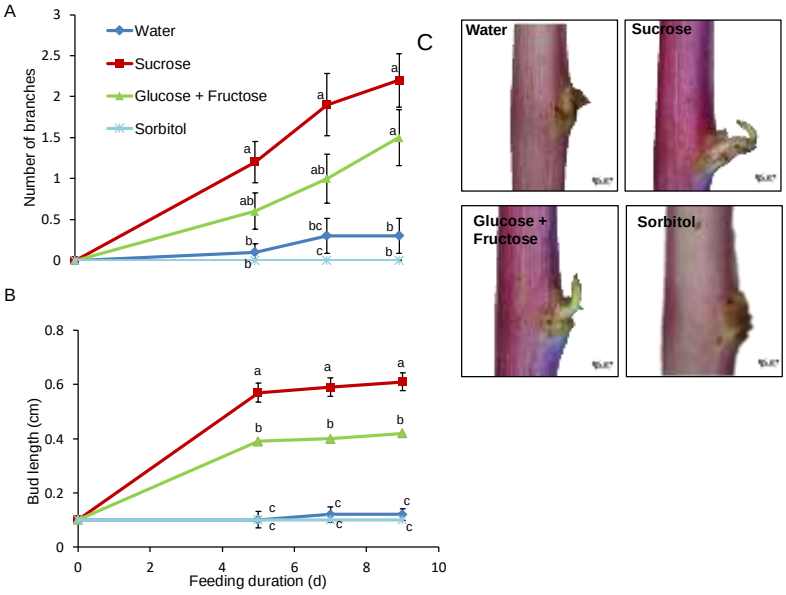


**Fig. 1.** Exogenous sucrose or hexoses induce lateral bud burst and elongation in etiolated stems. Sprouts were detached from the tubers and supplemented with sugars (sucrose, glucose + fructose, sorbitol, each at 300 mM) or water for nine days in the dark. **A,** Number of branches. **B,** Lateral bud length. **C,** Images showing the lateral node after 7 days of treatment. Bars = 100 µm. Results represent the mean of ten biological replicates. Error bars represent SE. Different letters indicate significant differences between treatments at each time point (one-way ANOVA, *p*-value < 0.05).

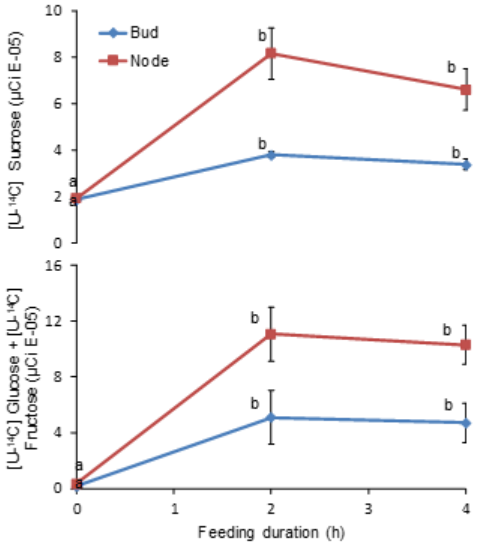


**Fig. 2**. Translocation of sucrose and hexoses from the stem into the lateral bud. Detached etiolated stems were fed with solution containing 1µCi [U-$^{14}$C] sucrose or 1µCi [U-$^{14}$C] glucose + [U-$^{14}$C] fructose for 0, 2 and 4 h under dark condition. Each value represents the mean of five independent measurements ± SE. Different letters represent significant differences between in time point in each treatment ($P < 0.05$).

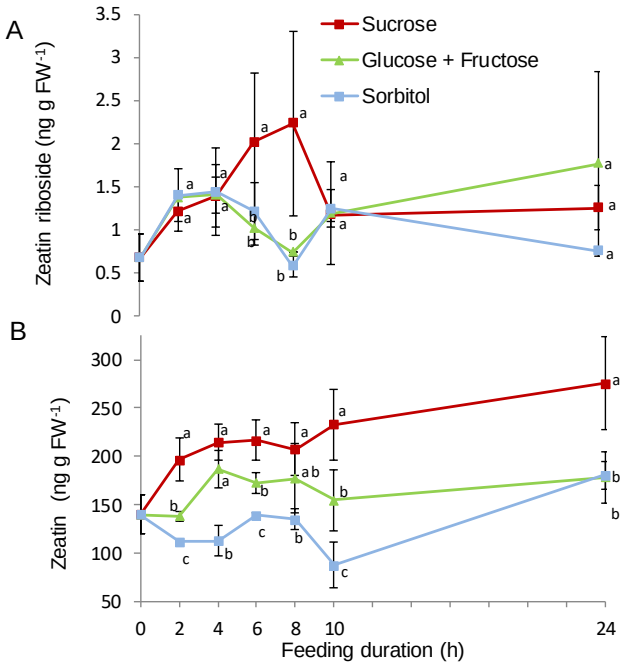


**Fig. 3.** Sucrose feeding of etiolated stems induces high level of endogenous cytokinin in the node of the lateral bud. Levels of **A,** zeatin riboside and **B,** zeatin in untreated sprouts (0 h) or sprouts supplemented with sugars (sucrose, glucose + fructose, sorbitol at 300 mM) or water, at different time intervals at 14°C, 95% relative humidity, in the dark. Results are means of three biological replicates. Error bars represent SE. Different letters indicate significant differences between treatments at each time point (one-way ANOVA, *p*-value < 0.05). FW, fresh weight.

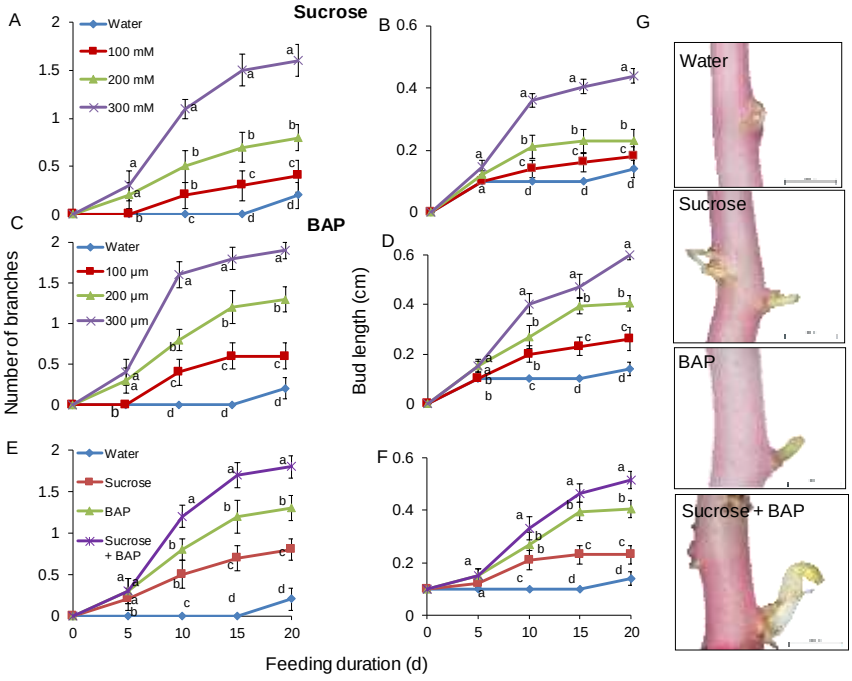


**Fig. 4.** Additive effect of sucrose and CK on etiolated stem branching and lateral bud elongation. Sprouts were detached from tubers, incubated at 14°C, 95% relative humidity, in the dark, and fed for 20 days with **A, B,** 0, 100, 200 or 300 mM sucrose; **C, D,** 0, 100, 200 or 300 µm BAP; **E, F,** 200 mM sucrose with or without 200 µm BAP, or water. The number of branches (**A, C, E**) and lateral bud length (**B, D F**) were recorded. **G,** Typical lateral buds after 15 days of feeding. Bars = 100 µm. Results are mean of ten biological replicates. Error bars represent SE. Different letters indicate significant differences between treatments at each time point (one-way ANOVA, *p*-value < 0.05).

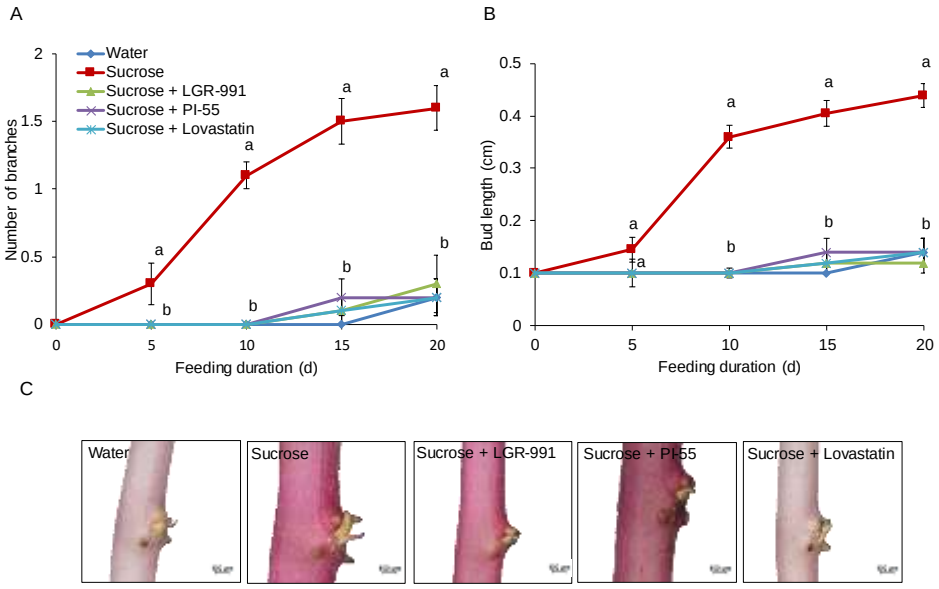


**Fig. 5.** Reduction of sucrose induced branching by CK inhibitors. Etiolated stems were detached from the tubers and fed with 300 mM sucrose, or 300 mM sucrose with CK-synthesis inhibitor (lovastatin, 200 µm), or with CK-perception inhibitors (LGR-991, PI-55, 200 µm), or water for 20 days at 14°C, 95% relative humidity, in the dark. **A,** Number of branches. **B,** Lateral bud length. **C,** Typical lateral buds after 15 days. Bars = 100 µm. Results are mean of ten biological replicates. Error bars represent SE. Different letters indicate significant differences between treatments at each time point (one-way ANOVA, *p*-value < 0.05).

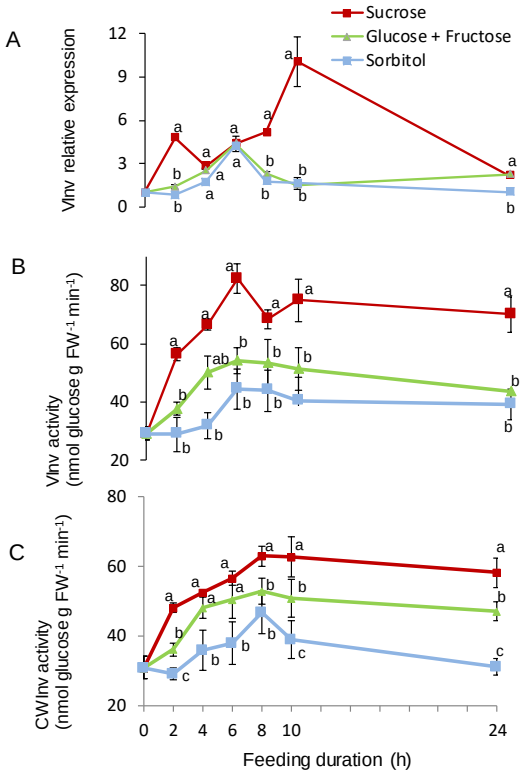


**Fig. 6.** Sucrose feeding of stems induces higher expression and activity of VInv in the lateral bud. Detached etiolated stems were fed with 300 mM sucrose, hexoses or sorbitol for 24 h in the dark. **A,** *VInv* transcript level at the lateral bud determined by real-time quantitative PCR. Gene transcript levels are expressed relative to controls (0 h) which were set to 1 and normalized to *Elf1* transcript level. **B, C,** Vacuolar invertase (VInv) and cell wall invertase (CWInv) activity at the stem node, respectively. Results are mean of three biological replicates. Error bars represent SE. Different letters indicate significant differences between treatments at each time point (one-way ANOVA, *p*-value < 0.05). FW, fresh weight.

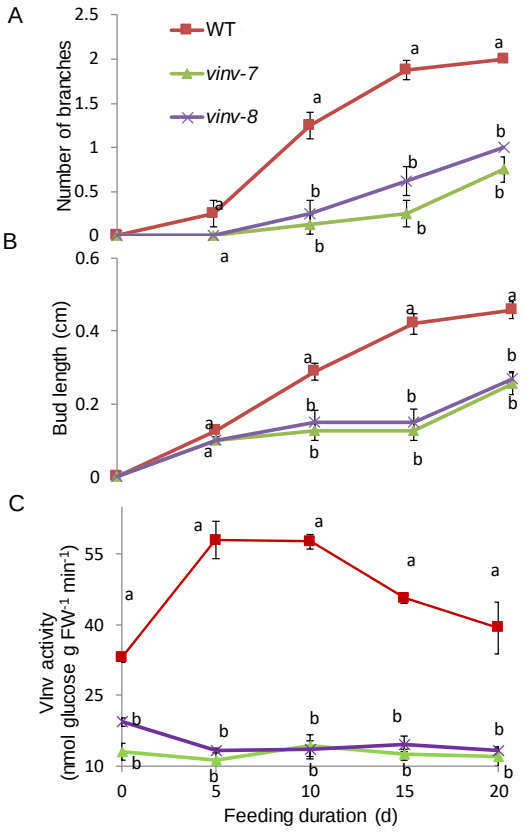


**Fig. 7.** Silencing of *VInv* reduces the effect of sucrose on stem branching. Detached etiolated stems of 'Desiree' (WT) and *VInv*-knockout lines (*vinv-7* and *vinv-8*) were fed with 300 mM sucrose for 20 days at 14°C, 95% relative humidity, in the dark. **A,** Number of branches. **B,** Lateral bud length. **C,** VInv activity at the stem node of the lateral bud. Results are mean of eight biological replicates. Error bars represent SE. Different letters represent significant differences between treatments at each time point (one-way ANOVA, *p*-value < 0.05). FW, fresh weight.

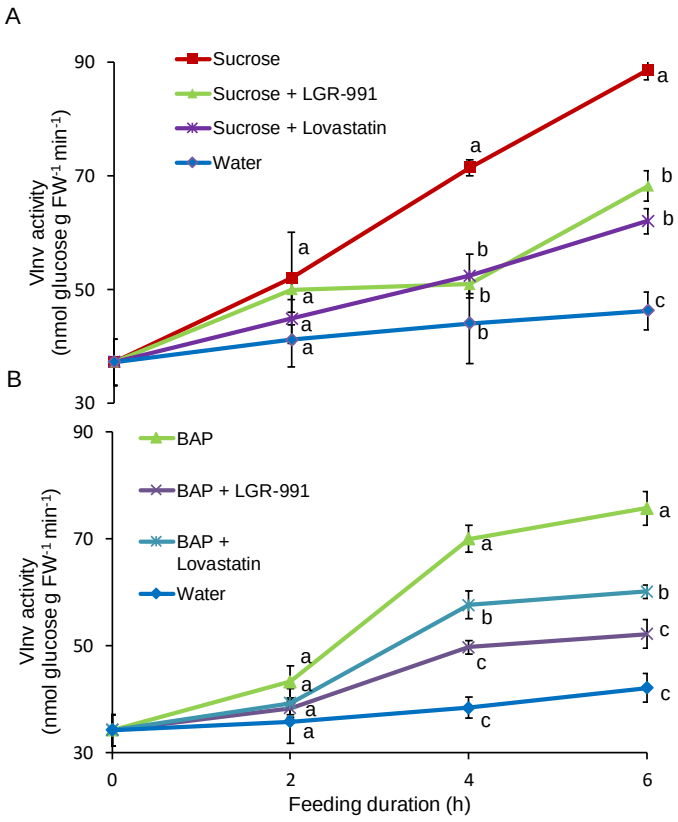


**Fig. 8.** CK inhibitors reduce VInv activity induced by sucrose or BAP. Detached etiolated stems were incubated for 6 h, in the dark and were fed with **A,** 300 mM sucrose, or 300 mM sucrose with CK-synthesis inhibitor (200 µm lovastatin) or with CK-perception inhibitor (200 µm LGR-991), or water, **B,** 200 µm BAP, BAP with CK-synthesis inhibitor (200 µm lovastatin), BAP with CK-perception inhibitor (200 µm LGR-991), or water. Results are mean of five biological replicates. Error bars represent SE. Different letters indicate significant differences between treatments at each time point (one-way ANOVA, *p*-value < 0.05). FW, fresh weight.

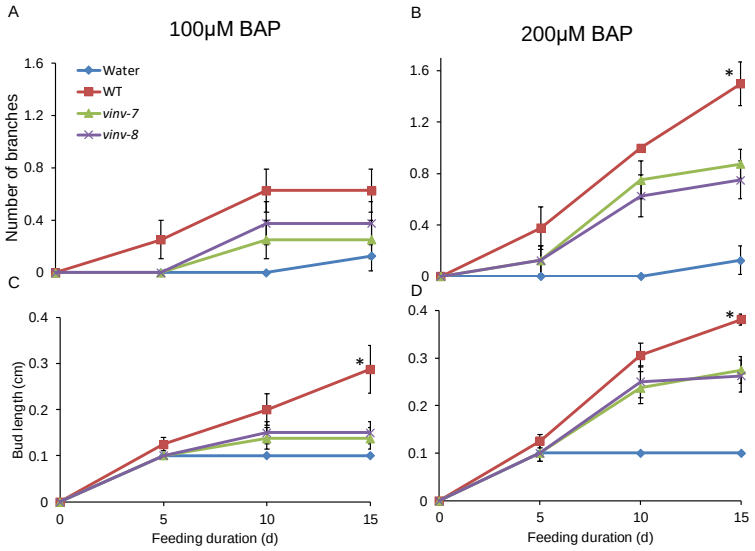


**Fig. 9.** Silencing of *VInv* reduces CK effect on stem branching. Etiolated stems were detached from tubers of WT (Désirée) and *VInv* mutants (*vinv-7* and *vinv-8*). The stems were supplied with 100 or 200 µM BAP or water for 15 days at 14°C in 95% relative humidity, in the dark. **A, B**, Number of branches. **C, D**, Lateral bud length. Results are mean of eight biological replicates. Error bars represent SE. * indicate significant differences between WT and each VInv mutant at each time point (Student's t-test, *p*-value < 0.05).

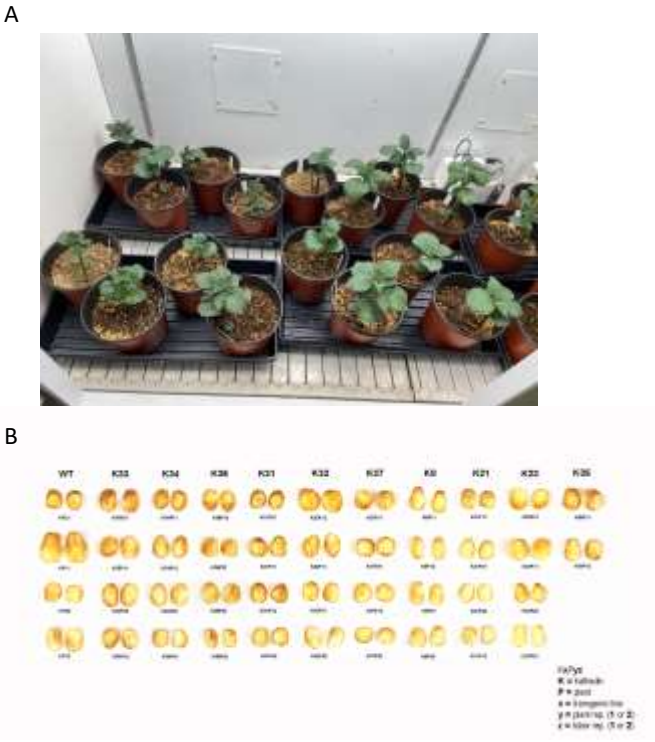


**Fig. 10.** Overexpression of *VInv* in the potato cultivar Katahdin and the effect on plant growth (A) and frying color of potato chips (B). Potato seedlings were grown in a growth chamber maintained at 16 h 24°C/8 h 16 °C light/dark. Overexpression lines show a similar phenotype with WT Katahdin plants. No dramatic color change was observed on chips processed from cold stored tubers (4°C for two weeks) from different overexpression lines.